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**Name of Journal:** *World Journal of Gastroenterology*

**Manuscript NO:** 114184

**Manuscript Type:** EDITORIAL

**Peroxiredoxin 1, pyroptosis, and the emerging frontier in colorectal cancer therapy**

Maurya DK. Prdx1 and pyroptosis in CRC therapy

## **Abstract**

Colorectal cancer (CRC) remains a major global health challenge, with high recurrence and mortality despite advances in surgery, chemotherapy, and immunotherapy. The study by He *et al* identifies a novel mechanism by which peroxiredoxin 1 (Prdx1) inhibits CRC progression through induction of pyroptosis, a pro-inflammatory form of programmed cell death. Traditionally viewed as an intracellular antioxidant that protects tumors from oxidative stress, Prdx1 assumes a paradoxical immunogenic role when released extracellularly as a damage-associated molecular pattern. Using patient samples, recombinant protein assays, and murine xenograft models, the authors demonstrate that Prdx1 activates the NOD-, LRR- and pyrin domain-containing protein 3 inflammasome/caspase-1/gasdermin D pathway, triggering membrane pore formation, tumor cell lysis, and release of interleukin-1 $\beta$ /interleukin-18. This cascade not only halts tumor proliferation, invasion, and migration but may also enhance anti-tumor immune surveillance. The study's strengths include rigorous mechanistic validation, clinical cohort data, inhibitor-based causal proof, and *in vivo* confirmation. However, questions remain regarding the upstream receptor for Prdx1, heterogeneity across CRC subtypes, and the balance between therapeutic benefit and inflammatory toxicity. By establishing Prdx1-induced pyroptosis as a driver of tumor suppression, this work advances a promising paradigm in CRC therapy, linking cell death to immune activation and pointing toward future biomarker-driven, pyroptosis-based interventions.

**Key Words:** Colorectal cancer; Peroxiredoxin 1; Pyroptosis; Damage-associated molecular pattern; Immunogenic cell death

Maurya DK. Peroxiredoxin 1, pyroptosis, and the emerging frontier in colorectal cancer therapy. *World J Gastroenterol* 2025; In press

**Core Tip:** Peroxiredoxin 1, long regarded as an intracellular antioxidant that protect tumors from oxidative stress, assumes a paradoxical role in colorectal cancer therapy. He *et al* reveal that extracellular peroxiredoxin 1 acts as a damage-associated molecular pattern, activating the NOD-, LRR- and pyrin domain-containing protein 3 inflammasome/caspase-1/gasdermin D pathway to trigger pyroptosis. This lytic, pro-inflammatory death suppresses colorectal cancer proliferation, invasion, and migration while amplifying interleukin-1 $\beta$ /interleukin-18-mediated immune surveillance. Using patient cohorts, recombinant protein assays, and xenograft models, the study rigorously validates this mechanism.

## INTRODUCTION

Colorectal cancer (CRC) represents one of the greatest ongoing challenges in oncology, not only due to its prevalence but also because of its lethality[1]. Globally, CRC accounts for nearly 10% of all cancer diagnoses and remains the second leading cause of cancer-related mortality, surpassed only by lung cancer[2,3]. Despite major improvements in diagnostic screening, surgical resection techniques, adjuvant chemotherapy protocols, and, more recently, targeted and immune-based therapies, CRC continues to present with high recurrence rates, therapy resistance, and metastasis-driven mortality[3]. Five-year survival rates drop dramatically in advanced stages, underscoring the urgent need for more effective and durable therapeutic interventions[4]. One reason for CRC's stubborn clinical trajectory lies in the complexity of its tumor microenvironment (TME). The TME is not a passive scaffold but an active determinant of disease outcome[5]. It is shaped by a network of cancer cells, fibroblasts, immune infiltrates, extracellular matrix components, and a milieu of soluble mediators. This ecosystem fosters chronic inflammation, angiogenesis, immune evasion, and metastatic capability[5]. Conventional therapies, although able to shrink tumors initially, often fail to reprogram the TME or overcome its inherent ability to suppress anti-tumor immune responses. Thus, strategies designed to trigger immunologically meaningful forms of cancer cell death are gaining traction. Among these, immunogenic

cell death (ICD) has emerged as a particularly promising mechanism[6]. Unlike classical apoptosis, which can be immunologically silent or even tolerogenic, ICD functions as a molecular alarm system. When tumor cells die *via* ICD, they expose and release damage-associated molecular patterns (DAMPs), which are then recognized by innate receptors on antigen-presenting cells[6]. This process not only alerts the immune system to danger but also facilitates adaptive immunity against the tumor. Well-studied DAMPs include calreticulin, which serves as an “eat me” signal, high mobility group box protein 1 (HMGB1), which promotes dendritic cell activation, and extracellular ATP, which acts as a chemoattractant for immune cells[6]. Collectively, these signals broadcast cellular stress or demise to the immune system, transforming death into an opportunity for host defense. Within this growing landscape, peroxiredoxin 1 (Prdx1) has recently entered the spotlight. Traditionally viewed as an intracellular antioxidant enzyme that maintains redox balance by scavenging peroxides, Prdx1 functions primarily as a cytoprotective factor. Elevated levels have often been associated with tumor progression, as cancer cells exploit its enzymatic role to withstand oxidative stress. However, emerging research has redefined Prdx1 as a context-dependent DAMP once released extracellularly, capable of stimulating innate immunity and inflammation[7]. This dual identity *i.e.*, guardian inside the cell, signaler when outside, makes Prdx1 particularly intriguing in cancer biology.

In his recent article, He *et al*[8] provide crucial insights into this paradox by demonstrating how recombinant Prdx1 (rPrdx1) fundamentally alters the trajectory of CRC cells. Their study establishes that rPrdx1 can suppress the malignant hallmarks of CRC *i.e.*, proliferation, invasion, and migration not by inducing apoptosis or necroptosis, but by triggering pyroptosis, a lytic and pro-inflammatory form of programmed cell death. Mechanistically, they show that this effect is mediated through <sup>3</sup> activation of the NOD-, LRR- and pyrin domain-containing protein 3 (NLRP3) inflammasome and subsequent cleavage of gasdermin D (GSDMD), the pore-forming executor of pyroptosis. Their finding is significant for two reasons. First, it highlights pyroptosis as a viable therapeutic axis in CRC, offering a means to terminate tumor

growth while simultaneously reshaping the immune response. Second, it places Prdx1, once considered merely a marker of oxidative stress at the crossroads of cancer cell death and immune activation. Far from being passive bystanders, DAMPs such as Prdx1 are increasingly recognized as drivers of therapeutic innovation, with the potential to convert “cold” colorectal tumors into immunologically “hot” tumors responsive to immunotherapy. Thus, the study by He *et al*[8] not only advances our understanding of CRC cell biology but also opens a new therapeutic avenue that intertwines tumor cell death, danger signaling, and immunoregulation. Prdx1-triggered pyroptosis represents a potential bridge between conventional cytotoxic strategies and next-generation immune-activating therapies, offering new hope in the ongoing fight against CRC.

### **PRDX1 IN CANCER**

<sup>2</sup> PRDXs, a family of thiol-dependent peroxidases composed of six PRDX isoforms (PRDX1 to PRDX6) which played important roles in anti-oxidation processes. The biology of Prdx1 illustrates the complexity and context-dependence of stress response proteins in cancer[9]. At its core, Prdx1 is a thioredoxin-dependent peroxidase, charged with reducing hydrogen peroxide and maintaining intracellular redox balance. This enzymatic antioxidant function is central to cellular survival, as unchecked oxidative stress can damage DNA, proteins, and lipids, leading to apoptosis or senescence. In healthy tissues, this role is protective, aligning Prdx1 with cytoprotection, tissue homeostasis, and defense against reactive oxygen species (ROS)-driven injury[10]. However, in the malignant state, where cancer cells hijack almost every biological system to enhance growth and evade death, this very antioxidant capacity becomes a tumor-enabling trait. Numerous studies across cancer biology have implicated elevated Prdx1 expression in tumor tissues[11]. For example, in hepatocellular carcinoma, overexpression correlates with worse prognosis and immune evasion; in breast cancer, Prdx1 has been linked to chemoresistance; in lung and prostate cancers, Prdx1 upregulation provides tumor cells with resilience against ROS generated by both

immune surveillance and therapeutic interventions such as radiotherapy or chemotherapy[11,12]. In these contexts, Prdx1 acts as a molecular shield, preserving cancer cell fitness under oxidative duress.

The paradox sharpens when Prdx1 crosses into the extracellular space. When released by necrotic or stressed cells, or through non-classical secretion mechanisms, extracellular Prdx1 functions as a DAMP, signaling “danger” to the immune system[13]. Binding to pattern recognition receptors such as Toll-like receptor 4 (TLR4) on immune or epithelial cells, Prdx1 can stimulate robust inflammatory signaling cascades. This shift turns a molecule of cellular maintenance into a prophet of immune alarm. In conditions such as sepsis and acute liver injury, extracellular Prdx1 has already been recognized as a mediator of sterile inflammation[7]. The study by He *et al*[8] leverages this paradox to interrogate CRC biology, effectively repurposing a normally intracellular antioxidant into an extracellular immunogenic effector. Their experiments elegantly illustrate that rPrdx1 is capable of inhibiting proliferation, migration, and invasion of CRC cell lines while actively inducing pyroptotic death. This is not mere toxicity; it is a specialized programmed lytic process mediated by inflammasome activation and GSDMD cleavage. Thus, a molecule commonly thought of as conferring tumor protection gains a radical new identity as a pro-death, anti-tumoral agent when freed from intracellular confines. The dual identity of Prdx1 underscores a broader principle in cancer biology: Molecules cannot be simplistically classified as “good” or “bad” for tumors. Their impact depends on context, subcellular localization, microenvironmental signals, and interaction with receptors. For clinicians and translational scientists, this presents both a challenge and an opportunity. On one hand, Prdx1’s protein nature complicates its candidacy as a universal biomarker; its elevation could either indicate tumor protection or, conversely, tumor vulnerability through immune activation. On the other, this very versatility could be harnessed therapeutically by selectively promoting extracellular or recombinant forms of Prdx1, it may be possible to flip a tumor-supportive molecule into a tumor-suppressive force. The Janus-faced behavior of Prdx1 thus aligns it with other molecules in oncology that

straddle dual roles such as HMGB1 or heat shock protein 90, which can both promote and inhibit tumorigenesis depending on context[14]. So, the lesson is clear: The functional trajectory of such molecules is dictated not merely by their intrinsic structure but by where, when, and how they act. In this light, Prdx1 emerges not simply as a passenger in redox biology but as a dynamic switch in cancer immunity, oscillating between protector and executioner. Understanding this dichotomy is critical, for it is in these dualities that novel therapeutic opportunities often lie.

### **PYROPTOSIS**

Among the diverse modes of programmed cell death, pyroptosis has garnered increasing attention in the past decade as both a cytotoxic and immunoregulatory process[15]. Unlike apoptosis, which is immunologically silent and characterized by controlled nuclear condensation and membrane blebbing, or necroptosis, which is a regulated necrosis driven by receptor-interacting protein kinase/mixed-lineage kinase domain-like signaling, pyroptosis is uniquely inflammatory in nature[15]. Its defining feature is the formation of plasma membrane pores that unleash a cascade of downstream biological consequences. Central to this process is the GSDM family of proteins, particularly GSDMD. Upon activation of upstream inflammasomes, caspase-1 cleaves GSDMD, liberating its N-terminal fragment, which oligomerizes to form pores within the cell membrane. This results in both catastrophic cell lysis and the release of pro-inflammatory cytokines, most notably interleukin (IL)-1 $\beta$  and IL-18[16]. The consequences of pyroptosis can be summarized into two interlinked outcomes. First, the direct elimination of tumor cells through lytic membrane rupture halts proliferation in a manner distinct from apoptosis, which demands intact intracellular organelles and energy reserves. Tumor cells succumb rapidly once membrane integrity collapses, preventing further invasion and colony formation. Second, and more importantly from an immunologic standpoint, pyroptosis orchestrates an alarm signal to the immune system. Through the release of DAMPs, as well as cytokines such as IL-1 $\beta$  and IL-18, pyroptotic cells shape the recruitment and activation of dendritic cells, macrophages,

and T lymphocytes. In the context of cancer, this can convert an immunologically “cold” microenvironment into a “hot” one, making tumors more susceptible to immune clearance or synergistic immunotherapy.

In his study, He *et al*[8] have makes a significant leap in connecting this paradigm to CRC. Deploying multiple assays such as transmission electron microscopy to capture hallmark pyroptotic morphology, lactate dehydrogenase assays to confirm membrane rupture, western blotting to document GSDMD cleavage, and cytokine release analyses to quantify IL-1 $\beta$ /IL-18, have provided solid evidence that rPrdx1, when applied extracellularly, induces pyroptosis in CRC cells. Crucially, they demonstrate that this is not a by-product of generalized cell stress leading to apoptosis or necroptosis. Supporting this conclusion, neither polyadenosine-diphosphate-ribose polymerase cleavage (apoptotic marker) nor mixed-lineage kinase domain-like phosphorylation (necroptosis marker) was observed; only pyroptotic features were present. In another study, Ye *et al*[17] have shown that the silencing of endogenous Prdx1 in oral squamous cell carcinoma <sup>1</sup> cells modulates the disease progression through the inhibition of autophagy *via* a ROS-independent mechanism, promotion of pyroptosis *via* a ROS-dependent mechanism, and involvement in the intricate interplay between autophagy and pyroptosis. <sup>1</sup> rPrdx1 regulated pyroptosis in a ROS-dependent way and modulated autophagy in a ROS-independent way, involving the crosstalk between pyroptosis and autophagy. Perhaps <sup>1</sup> the most elegant aspect of the study lies in its mechanistic precision. By employing pharmacologic inhibitors, the researchers could dissect the pathway: MCC950, a well-characterized NLRP3 inflammasome inhibitor, and Ac-FLTD-CMK, a GSDMD-specific inhibitor, each abrogated pyroptosis induced by rPrdx1. When these inhibitors were applied, CRC cells not only survived but also retained their proliferative and migratory capacities, underscoring the centrality of the NLRP3/caspase-1/GSDMD pathway. This pathway’s specificity was further validated by showing that alternative executors such as GSDME or caspase-4/5 (non-canonical inflammasome axis) were not activated. Additionally, the study highlighted cell-line specificity. While RKO and SW480 cells underwent pyroptosis upon rPrdx1 stimulation,

HCT116 cells were resistant. Mechanistically, this resistance correlated with the absence of GSDMD expression in HCT116 cells. This observation underscores a central caveat for pyroptosis-based therapies: Tumor heterogeneity matters. The efficacy of such strategies will likely hinge on intrinsic expression of GSDMs or inflammasome components, positioning GSDMD itself as a predictive biomarker for response. Finally, the functional consequences of rPrdx1-triggered pyroptosis extend beyond cell culture. *In vivo* murine xenograft models demonstrated reduced tumor burden with systemic rPrdx1 administration.

### **STRENGTHS AND LIMITATIONS OF THE STUDY**

A central strength of the work done by He *et al*[8] lies in its methodological comprehensiveness. Too often, studies exploring novel cell death pathways rely on a limited set of readouts that risk conflating mechanisms, especially since apoptosis, necroptosis, ferroptosis, and pyroptosis can share morphological or biochemical overlaps[8]. Here, the investigators employed a multimodal analytic strategy encompassing structural, biochemical, and molecular analyses, thereby triangulating evidence for pyroptosis. The use of transmission electron microscopy provided classic ultrastructural confirmation of pyroptotic morphology, including membrane pore formation distinct from apoptotic nuclear changes. Parallel lactate dehydrogenase assays quantified membrane rupture, while western blotting confirmed cleavage of GSDMD, and enzyme-linked immunosorbent assay measurements of IL-1 $\beta$ /IL-18 verified functional cytokine release. Such integration across platforms not only strengthens confidence in their conclusion but also raises the bar for rigorous mechanistic cancer biology. Another commendable strength was the incorporation of *in vivo* validation. Many promising cell death discoveries falter because they remain confined to immortalized cell line models, without attention to the complexities of whole-organism physiology. By demonstrating that systemic delivery of rPrdx1 suppressed tumor growth in xenograft mice, the authors bridge a critical translational gap. Although these murine models are not immune-competent, they nonetheless

underscore therapeutic feasibility and justify more advanced preclinical studies. Equally noteworthy was the use of pathway-specific inhibitors. By systematically applying the NLRP3 inflammasome inhibitor MCC950 and the GSDMD inhibitor Ac-FLTD-CMK, the team went beyond correlative association to demonstrate causal involvement of these molecules. Their inhibition abrogated pyroptosis, confirming mechanistic specificity. Such pharmacologic dissection lends particular weight to claims of pathway dependency and distinguishes this study from more descriptive reports. Finally, a pragmatic strength rests in the inclusion of clinical samples from 60 CRC patients. Too often, laboratory discoveries run the risk of irrelevance if not tethered to patient-based biology. By confirming that Prdx1 was indeed upregulated in human CRC tissues compared with adjacent normal, the study demonstrates clinical anchoring and provides biomarkers that can be interrogated against therapeutic response or prognosis in future cohorts.

DAMPs, such as HMGB1 and ATP, play critical roles in CRC pathogenesis and immune modulation. Where, HMGB1 is a nuclear protein released upon cell damage, acts as an extracellular signal that triggers inflammation by binding to receptors like receptor for advanced glycation end product and TLR4, promoting tumor cell proliferation, angiogenesis, and immune evasion in CRC. Elevated HMGB1 levels in CRC tissues are linked to poor prognosis and enhanced metastatic potential, as this protein stimulates the release of pro-inflammatory cytokines, recruits myeloid-derived suppressor cells, and fosters an immunosuppressive microenvironment. Similarly, extracellular ATP acts as a signaling molecule released by dying cells and binds to purinergic receptors on immune and tumor cells, influencing CRC progression. ATP can activate dendritic cells and induce ICD, potentially boosting anti-tumor immune responses. However, persistent ATP signaling may also promote inflammation, tissue repair, and resistance to apoptosis, contributing to tumor growth and therapy resistance in CRC. So these DAMPs alongside Prdx1 establishes a broader framework showing how different DAMPs orchestrate inflammation and tumor dynamics in CRC.

Despite the many strengths, some caveats temper the enthusiasm. Foremost is the absence of clarity regarding the cell-surface receptor mediating Prdx1 recognition in CRC. While activation of the NLRP3 inflammasome and downstream caspase-1/GSDMD is firmly established, the initial trigger remains undefined. Past literature implicates TLR4 as a plausible mediator in macrophages, but it remains unproven in epithelial cancer cells. Identifying this receptor will be essential to design targeted strategies that selectively exploit Prdx1's pyroptotic function. Another limitation is the restricted spectrum of tested cell lines. Only three CRC models were studied, with robust effects observed in RKO and SW480 cells but not in HCT116. This divergence, attributed to cell line-specific absence of GSDMD, highlights CRC heterogeneity. Since colorectal tumors exhibit vast genetic, epigenetic, and immunologic diversity, larger panels - ideally incorporating patient-derived organoids or xenografts, must be examined to establish generalizability. Equally important is the lack of immune microenvironment analysis. Pyroptosis is not only a cell-death mechanism but also an immune-modulatory process. By releasing IL-1 $\beta$  and IL-18, pyroptotic cancer cells might recruit cytotoxic lymphocytes, reshape macrophage polarization, and enhance antigen presentation. These dynamics cannot be captured in immunodeficient murine xenograft models. Confirming that pyroptosis translates into durable anti-tumor immunity requires immune-competent models. Lastly, there is the inflammation conundrum. While pyroptosis can boost immune attack against tumors, it may also unleash systemic inflammation with detrimental consequences. Excessive IL-1 $\beta$  and IL-18 can exacerbate cachexia, colitis, or systemic cytokine toxicity. Thus, any therapeutic development of rPrdx1 must carefully evaluate inflammatory safety profiles, dose windows, and delivery methods to prevent harm.

### **FUTURE DIRECTIONS**

Identifying the specific receptor responsible for Prdx1 signaling in CRC cells, whether TLR4, receptor for advanced glycation end product, or absent in melanoma 2, will illuminate the first step of the pathway. Understanding how macrophages, T cells, and

dendritic cells interact with pyroptotic CRC cells is equally vital and requires immune-competent mouse models. Combination regimens should explore whether rPrdx1 augments checkpoint blockade, leveraging IL-1 $\beta$ /IL-18 signaling to recruit T-cell responses. Future research should investigate the mechanistic interplay between rPrdx1 and checkpoint pathways, measure clinical outcomes like progression-free survival, and assess changes in immune cell infiltration and cytokine profiles. The rationale is that rPrdx1 may synergize with programmed death protein 1 (PD-1)/programmed death-ligand 1 (PD-L1) inhibitors by modulating the tumor immune microenvironment, boosting effector T cell activation and potentially reversing immune suppression mediated by DAMPs in CRC. Specifically, studies may evaluate rPrdx1 as an adjunct with PD-1 (*e.g.*, pembrolizumab, nivolumab) and PD-L1 (*e.g.*, atezolizumab, durvalumab) inhibitors in both preclinical CRC models and biomarker-stratified patient cohorts, aligning with biomarker-driven precision oncology. The systemic risks of inflammation must also be carefully mapped. Since rPrdx1 could aggravate colitis or systemic toxicity, targeted delivery strategies such as nanoparticle encapsulation, antibody conjugation, or local administration should be developed. Finally, clinical observational studies should explore GSDMD as a prognostic biomarker, stratifying patients likely to respond and guiding patient selection.

## **CONCLUSION**

The discovery that Prdx1 induces CRC cell pyroptosis through NLRP3/GSDMD activation provides a significant conceptual and translational advance. While questions regarding upstream receptors, immune integration, and clinical safety remain, this study highlights the therapeutic potential of harnessing pyroptosis for cancer therapy. With growing interest in the crosstalk between cell death and immunogenicity, the work of He *et al*[8] adds momentum to a field poised to redefine approaches to refractory malignancies, particularly CRC. Once dismissed as a catastrophic inflammatory process, pyroptosis is now being reimaged as a precision tool in onco-immunology, emphasizing the promise of “precision pyroptosis therapy”. This strategy

leverages the targeted induction of inflammatory cell death based on specific biomarkers such as Prdx1 and other DAMP-related pathways. Such an approach exemplifies the paradigm of biomarker-driven personalized medicine, potentially enhancing treatment specificity and efficacy in CRC patients by modulating DAMP-mediated immune and inflammatory responses. The future may see oncologists deliberately “igniting” tumor cells from within, turning Prdx1 from a biomarker of stress into a weapon of therapeutic intent.

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